

Article

# Modern Analogue Approach Applied to High-Resolution Varved Sediments—A Synthesis for Lake Montcortès (Central Pyrenees)

Teresa Vegas-Vilarrúbia <sup>1,\*</sup>, Valentí Rull <sup>2</sup>, Maria del Carmen Trapote <sup>1,2</sup>, Min Cao <sup>3</sup>, Antoni Rosell-Melé <sup>3,4</sup>, Teresa Buchaca <sup>5</sup>, Joan Gomà <sup>1</sup>, Pilar López <sup>1</sup>, Javier Sigró <sup>6</sup>, Elisabet Safont <sup>1</sup>, Núria Cañellas <sup>2</sup>, Sandra Garcés-Pastor <sup>1</sup>, Santiago Giralt <sup>2</sup>, Juan Pablo Corella <sup>7</sup> and Núria Pérez-Zanón <sup>6</sup>

<sup>1</sup> Faculty of Biology, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain; mctrapote84@gmail.com (M.d.C.T.); jgoma@ub.edu (J.G.); marilopez@ub.edu (P.L.); esafont@ub.edu (E.S.); sgarcespastor@gmail.com (S.G.-P.)

<sup>2</sup> Institute of Earth Sciences Jaume Almera (ICTJA-CSIC), C. Lluís Sole i Sabarís s/n, 08028 Barcelona, Spain; vrull@ictja.csic.es (V.R.); nuriacatcb@gmail.com (N.C.); sgiralt@ictja.csic.es (S.G.)

<sup>3</sup> Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), Universitat Autònoma de Barcelona, Bellaterra, 08193 Catalonia, Spain; smilecaomina@hotmail.com (M.C.); antoni.rosell@uab.cat (A.R.-M.)

<sup>4</sup> Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, 08010 Catalonia, Spain

<sup>5</sup> Centre for Advanced Studies of Blanes (CEAB-CSIC), Accés a la Cala St. Francesc 14, 17300 Blanes, Spain; buch@ceab.csic.es

<sup>6</sup> Center for Climate Change (C3), Universitat Rovira i Virgili, C/ Joanot Martorell 15, Vila-seca, 43500 Tarragona, Spain; javier.sigro@urv.cat (J.S.); nuria.perez@bsc.es (N.P.-Z.)

<sup>7</sup> Université Grenoble Alpes, CNRS, IRD, Grenoble INP, IGE, 38000 Grenoble, France; pcorella@iqfe.csic.es

\* Correspondence: tvegas@ub.edu

Academic Editors: Bernd Zolitschka and Matthew Peros

Received: 21 May 2019; Accepted: 19 December 2019; Published: 2 January 2020



**Abstract:** In Quaternary paleosciences, the rationale behind analogical inference presupposes that former processes can be explained by causes operating now, although their intensity and rates can vary through time. In this paper we synthesised the results of different modern analogue studies performed in a varved lake. We discuss their potential value to obtain best results from high resolution past records. Different biogeochemical contemporary processes revealed seasonality and year-to-year variability, e.g., calcite precipitation, lake oxygenation, production and deposition of pollen and phytoplankton growth. Fingerprints of the first two of these processes were clearly evidenced in the varve-sublayers and allow understanding related to past events. Pollen studies suggested the possibility of identifying and characterizing seasonal layers even in the absence of varves. Marker pigments in the water column were tightly associated with phytoplankton groups living today; most of them were identified in the sediment record as well. We observed that 50% of these marker pigments were destroyed between deposition and permanent burying. In another study, seasonality in the production/distribution of branched glycerol dialkyl glycerol tetraethers (brGDGTs) and derived temperature estimates were investigated in catchment soils and particles settling in the lake. The signatures of brGDGTs in depositional environments mainly were representative of stable conditions of soils in the catchment that last over decades; no brGDGTs seemed to be produced within the lake. The main contribution of this review is to show the advantages and limitations of a multiproxy modern-analogue approach in Lake Montcortès as a case study and proposing new working hypotheses for future research.

**Keywords:** endogenic varves; calcite precipitation; pollen traps; meromixis; freshwater GDGTs; pigments; long-term ecology

## 1. Introduction

Analogy is a powerful form of reasoning that allows two domains to be considered as similar, based on their relationships [1–3]. The analogue approach permits the extension of knowledge of a base domain to a target past domain by virtue of their expected similarity and by means of analogical inference. This process involves reproducing structures and processes from the base to the target in which missing information is generated. Nonetheless, constraints must be placed on what information is to be carried over [4], in order to obtain inferences that are as realistic as possible. In Quaternary paleosciences, the rationale behind analogue inferences is based on postulating that past natural changes can be explained by causes operating today (principle of uniformitarianism), but that intensity and rates at which processes take place vary through time [5]. On the other hand, reconstruction and derived inferences for any proxy (natural recorders of variability) demand a thorough comprehension of the spatial and temporal frame in which processes and environmental conditions leading to the existence and persistence of each particular proxy make sense.

Examples of modern analogue studies of proxies and their applications are widespread. In paleoecology, fossil assemblages (pollen, diatoms, pigments, ostracods etc.) are examined for coincidences with modern assemblages, and the direction and rate at which communities vary through space and time can be measured quantitatively by a wide array of numerical approaches and techniques [6–8]. For instance, in the Peruvian Andes, the pollen/altitude relationship in a forest transect was studied by means of correspondence analysis, and the good statistical correlation between altitude and the first component allowed building a transfer function with statistical reliability to infer altitude from pollen data [9]. In another case, a surface-sediment survey of pigments in 100 lakes (Northern Sweden) was combined with a reconstruction of sedimentary pigments from Lake Seukokjaure. Light regime and terrestrial supplies of  $^{13}\text{C}$ -depleted carbon turned out to be important controls of primary production, whereas depth, elevation and conductivity discriminated between different phototrophic communities. The application of these results to the sediment record demonstrated the importance of terrestrial vegetation for the development of the lake, and of specific factors to control algal community composition, across the Holocene [10]. The modern analogue approach can be used to build models for climate change reconstruction. Central to these models is the development of transfer functions that, by definition, are “empirically derived equations for calculating quantitative estimates of past atmospheric or oceanic conditions from paleontological data” [11]. Transfer functions have been widely applied during the last decades. For example, in Patagonia, chironomids of surface sediment of 63 lakes were used to model the atmospheric annual average temperature. The good correlation between estimated and measured temperatures allowed applying the model to the chironomid record of Lake Potrok Aike. The reconstructed trends of temperature were consistent with other quantitative records of the Southern Hemisphere [12]. In the Pyrenees, present chrysophyte cysts distribution is influenced by altitude, and a transfer function was developed to assess the local “altitude anomaly” on a lake site throughout time. A warming trend was detected showing sub-millennial climatic variability from the early Holocene to 4 kiloyears (kyr) before present (BP) [13]. Sometimes, finding modern analogues is not straightforward. For example, a modern analogue study of the Otter lake helped understand why and how siderite ( $\text{FeCO}_3$ ) accumulated only in varved sediments older than 1200 calibrated years, even though limnological data did not evidence siderite precipitation. The authors combined modern data with siderite time series, abundances of major element in sediment and experimental results on siderite precipitation. They showed that the lake system alternated between ferruginous, meromictic phases with intense precipitation of siderite and holomictic phases with very low siderite precipitation [14].

Temporal resolution that can be achieved with non-laminated sediment is often constrained to more than decadal resolution. Reconstructions of environmental variability at high resolution scales using varved sediments have provided an unprecedented level of detail that needs to be properly extracted and interpreted [15]. This implies knowledge of fine-tuned mechanisms that need to be observed today at the same temporal resolution as they occur in past records [16]. Lakes with varved sediments are especially well suited for paleoecological and paleoclimatic research demanding a high

level of detail, as it is possible to downscale to annual and seasonal resolution [15,17]. The interpretative power of such high-resolution paleoenvironmental reconstructions depends on the availability of suitable modern analogues with the same temporal resolution [16]. With the advent of new techniques and proxies that allow acquiring an increased level of detail, modern analogue studies are gaining importance, a fact we want to highlight with this paper.

We have taken advantage of the varved sediments of the Mediterranean Lake Montcortès and conducted several independent modern analogue studies. Our aim was to document the potential of the modern analogue approach not only by determining how similar modern and past communities are, but also by investigating different contemporary biological and physico-chemical processes that are expected to also have occurred in the past on annual and seasonal time scales. We also explored which factors and relationships between modern and subfossil proxies can limit or hinder the applicability of the modern analogue approach representing a potential source of error in analogue analysis. Such errors should be foreseen and minimised [6]. In this context, we examined how the different modern analogue approaches can help extract important cause-effect relationships at seasonal, annual and subdecadal time scale. We discussed, evaluated and synthesised the published results of four modern analogue studies carried out for Lake Montcortès:

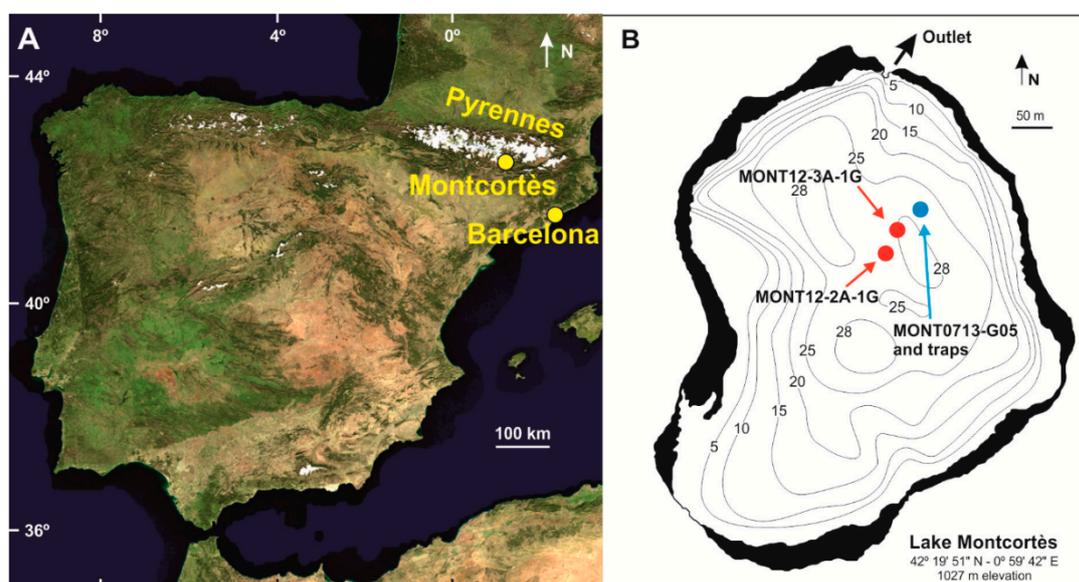
- (1) Relationships between calcite precipitation, environmental variables and primary producers and their influences on the thickness and patterns of calcareous varve sublayers were examined as high-resolution paleoclimate indicators [18].
- (2) Seasonal pollen sedimentation was compared with local meteorological variables to determine whether contemporary seasonal deposition is consistent with the two layers of the varve pattern archived in the sediments [19].
- (3) Contemporary shifts in dissolved O<sub>2</sub>, Fe, Mn and anaerobic phototrophic bacteria markers were assessed, in order to investigate whether mixing and oxygenation dynamics Lake Montcortès have been the same over the last five centuries [20].
- (4) Distribution of branched glycerol dialkyl glycerol tetraethers (GDGTs) in settling particulate matter and catchment soils was analysed in order to determine whether branched glycerol dialkyl glycerol tetraethers (brGDGTs) are seasonally biased in environments where annual brGDGTs production may not be constant [21].

Additionally, we provided and discussed the first results from ongoing research that aimed at relating contemporary phytoplankton taxa in Lake Montcortès to their corresponding marker pigments, and then to subfossil pigment records at subdecadal time scales.

## 2. Environmental Settings

Lake Montcortès (Figure 1) belongs to the southern flank of the Central Pyrenees (Catalonia, Spain) at 42°19'N, 0°59'E and 1027 m altitude (Figure 1). This karstic lake located on carbonated and evaporitic Mesozoic substrate is chiefly characterised by Triassic limestones, marls and evaporites, as well as Oligocene-carbonate conglomerates [22,23]. Climatic data collected at La Pobla de Segur meteorological station show that the annual average air temperature is 12.8 °C (2.9 °C in January to 23.2 °C in July). Total annual precipitation is 669 mm (33.4 mm in February and 88.4 mm in May). Maximum and minimum annual air temperatures were 41 and −20 °C, respectively. The vegetation of the catchment area is evergreen with deciduous oak forests (*Quercus rotundifolia* and *Q. pubescens*), conifer forests (*Pinus nigra*), pastures and crops. The vegetation belt at the shore is dominated by hygrophite communities of *Typha domingensis* or *Cladium mariscus*, reed beds of *Phragmites australis* and communities of *Carex riparia*. Rush formations and grasslands grow on occasionally flooded soils [24]. The lake surface area is 0.14 km<sup>2</sup> and its maximum water depth 32 m [25]. Only an ephemeral inlet exists, but water is drained by an outlet located at the northern shore. The lake's sedimentary record shows thin, well preserved biogenic varves formed during the Late Holocene. The varve structure

alternates between sublayers of endogenic calcite and organic detritus with additional detrital layers and turbidites embedded in the varve succession [22,26,27].



**Figure 1.** (A) Location of Lake Montcortès in the Pyrenean mountain range (NE Iberian Peninsula). (B) Bathymetric map with position of the cores and the traps.

### 3. Materials and Methods

Here, we provided a summary of the main field and laboratory methods used. More detailed information can be found in corresponding publications and literature therein [18–20].

#### 3.1. Monitoring and Coring

The limnological characteristics of Lake Montcortès were assessed monthly from October 2013 to October 2015. Detailed vertical profiles of temperature (T), dissolved oxygen (DO), electric conductivity (EC) and pH were obtained using a [28,29] multiparametric datasonde Hydrolab DS5. Water samples were obtained with an adapted submersible bilge pump at three depths in the epi-, meta- and hypolimnion for alkalinity, cations, total phosphorus (TP), total nitrogen (TN), suspended solids and molecular marker analyses (pigments and brGDGTs), and at two depths (epi- and metalimnion) for phytoplankton microscopic examination. Between 1.5 and 3 L of water was filtered through Whatman GF/F glass-fibre filters for pigment analysis. These filters were protected with aluminium foil and frozen to prevent degradation until laboratory analysis.

A set of sediment traps was deployed at 20 m water depth from a floating platform located at the deepest part of the lake, coinciding with the site where sediment coring was performed. Total suspended solids, calcite crystals [18], pollen and spores [19], marker pigments and settling particulate matter for brGDGT extractions [20] were collected in these traps, with monthly or quarterly (three months) frequency depending on the variable of interest. Additionally, replicated topsoil samples from a depth of 2–5 cm were collected monthly for brGDGT analysis at three sites within a 10 m radius in the catchment area. These samples were combined to reduce soil heterogeneity.

Two UWITEC gravity cores (MON12-3A-1G, 78 cm length, and MON12-2A-1G, 106 cm length) were retrieved at the deepest area of the lake basin (30 m depth) at the same location of the sediment trap (Figure 1) [27]. The varve chronology was performed by double counting in 14 overlapping thin sections. Only 1% of varves were interpolated due to poor varve preservation [27]. Two independent dating methods using AMS  $^{14}\text{C}$  and  $^{210}\text{Pb}$  radiometric dating techniques were previously achieved in the Lake Montcortès sedimentary sequence [22,26,27]. The agreement between these independent chronologies supports the robustness of the varve chronology.

### 3.2. Laboratory Analyses

The calcite saturation index ( $\Omega$ ) was estimated from carbonate and calcium concentrations.  $\text{Ca}^{2+}$  was measured with inductively coupled plasma atomic emission spectroscopy, and  $\text{CO}_3^{2-}$  was calculated from alkalinity, pH and temperature with the CO2SYS\_XLS v 2.1 program [28]. To confirm the presence of calcite crystals and to assess seasonal variations of their size and shape, trapped material from traps was analysed by scanning electron microscopy. Total suspended solids retained in the traps were analysed for total particulate carbon (TPC) and total particulate organic carbon (POC) with an elemental organic analyser, Thermo EA 1108.

Diatom specimens of quarterly sediment traps were prepared, identified and counted using standard methods [29]. A minimum of 300 valves were counted per sample; identification was carried out following Krammer and Lange-Bertalot [30,31]. Epilimnetic and metalimnetic phytoplankton was settled using sedimentation chambers; quantification was performed applying Utermöhl's technique for counting combined with biovolume conversion as  $\text{mm}^3 \text{L}^{-1}$ . Analysis of samples for pollen analysis followed standard methods [32] after addition of exotic *Lycopodium* spores. Pollen sum included all pollen types except the superabundant *Pinus* and *Quercus*. See the original palynological work for more detailed information on chemical processing, counting and plotting/statistical procedures [11]. As in earlier studies [33–35], identification of pollen was based on our own general reference collection for the Pyrenees, enriched after detailed floristic and vegetation studies around Lake Montcortès area [24].

Geochemical data to trace high resolution oxic/anoxic events of the hypolimnion were obtained on core MON12-3A-1K by X-ray fluorescence (XRF) analysis (2000 A, 10–30 kV and 20–50 s measuring time) (0.2 mm resolution) using an XRF core-scanner (Avaatech Analytical X-Ray Technology, Netherlands). We obtained sub-annual resolution time series for Fe, Mn, Ca, Si, S, Ti and Br. The former five elements were normalised to Ti. The Fe/Mn ratio was used as the main redox tracer, together with marker pigments of sulphur bacteria, such as okenone (oken) and isorenieratene (isore), which gave information at the subdecadal level.

A second core (MONT-0713-G05) was used for pigment analyses; sediment samples were obtained at sampling intervals of 5 mm, reaching subdecadal resolution, the frozen sediment samples were freeze dried before analysis. Pigment analyses were also performed on epi-, meta- and hypolimnetic water samples and sediment trap material collected in Whatman GF/F glass fibre filters. Marker pigments were extracted from frozen filters or freeze-dried sediment in 5 ml of 90% acetone. The extract was centrifuged, filtered and analysed with ultra-high-performance-liquid-chromatography following the method of [36] with some modifications. Pigments were identified by evaluating their retention times and absorption spectra against a library based on algae and photosynthetic bacterial cultures [37].

To isolate GDGTs, soil samples and filters from sediment traps were extracted monthly, evaporated and then fractionated over an aminopropyl silica column. The fractions containing the lipids were dried, re-dissolved and filtered. brGDGTs were analysed by high-performance liquid chromatography coupled with mass spectrometers and detected in selected ion monitoring modes; prior to extraction, a synthetic tetraether lipid with a structure typical of neutral archaeal membrane lipids was added to the sample. Further analyses in soils were performed for water content and total organic carbon (TOC).

## 4. Results

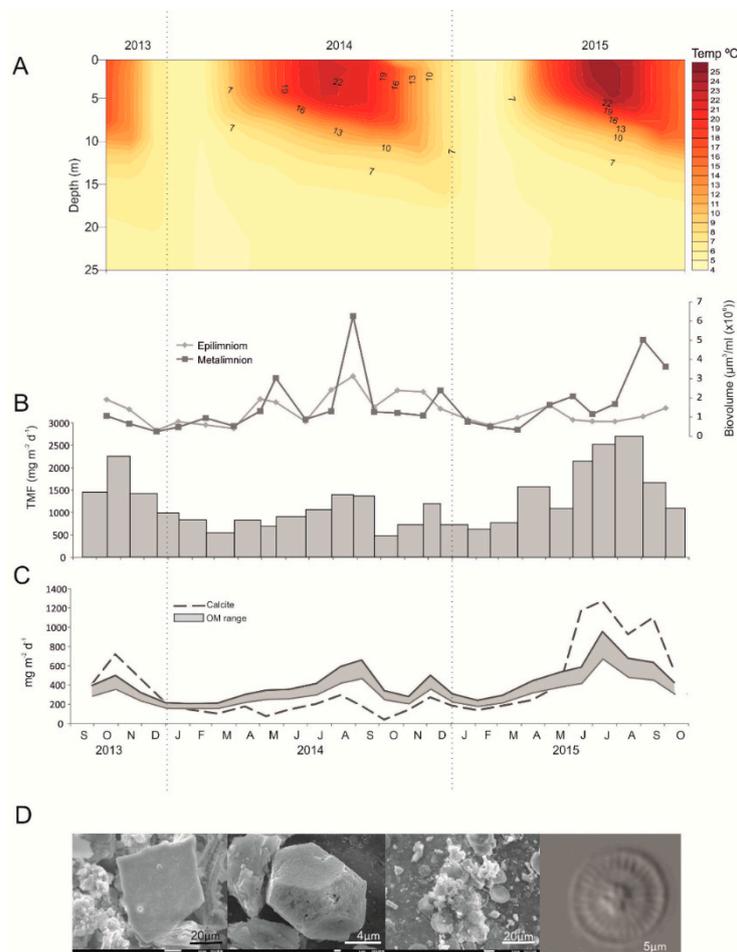
### 4.1. Biogeochemical Processes Involved in Varve Formation: Where, When, and How?

Processes driving varve formation are remarkably variable and site-specific. Consequently, detailed knowledge of the local processes that promote and modulate particle flux dynamics to the sediment for each site becomes essential. This knowledge is a pre-requisite for proper paleoenvironmental and paleoclimatic interpretations of the sedimentary archive [17,38] and for building reliable varve-based chronologies. An independent absolute varve chronology was obtained from the composite sedimentary sequence of both sediment cores extending from 2012 back to Common Era (CE) 1347 [27]. The varves

of Lake Montcortès are formed by couplets of white calcite and brownish organic layers. The white layer was assumed to have deposited during spring/summer and the dark layer in fall/winter [17,27]. Biogenic varve formation is highly complex as it adds physical and chemical processes to the complex biological processes involved [15].

With this modern analogue study several goals are pursued for Lake Montcortès: (i) to identify the main limnological and sedimentological mechanisms leading to varves formation; (ii) to establish in which season(s) the deposition of individual calcite sub-laminae occurs and (iii) to determine if the obtained results are coherent with the varve structure and previously proposed mechanisms of their formation [26].

Elevated  $\Omega$  values were recorded in the epi- and metalimnion Lake Montcortès from late spring to early fall, suggesting that calcite precipitation mostly occurred during this period. The deposition rates were highest in summer when photosynthetic organisms were most active (Figure 2A,B). Additionally, higher POC values were observed during this period, indicating that the main origin of calcite crystals were newly formed autochthonous  $\text{CaCO}_3$  precipitates (Figure 2C).



**Figure 2.** Summary of monthly water column and sediment trap monitoring. (A) Water column temperature at 1 m resolution. (B) Total mass fluxes (TMF) of monthly sediment traps compared with the phytoplankton biovolume of epi- and metalimnion. (C) Calcite fluxes versus organic matter (OM) fluxes of sediment trap material. (D) Well-formed blocky and rhombohedral calcite crystals, calcite crystals and diatom frustules and one of the small centric diatoms found in sediment trap material. Redrawn and modified from [18].

In fact, the presence of blocky, polyhedral calcite crystals was confirmed in all sediment trap samples and showed a clear seasonal pattern, being smaller when formed in spring and summer

(Figure 2D). Deposition of smaller crystals was not only concurrent with  $\Omega$ , but also with larger fluxes of very tiny diatoms ( $\sim 10 \mu\text{m}$ ), suggesting that phytoplankton cells could be acting as calcite condensation nuclei under enhanced calcite  $\Omega$ , pH and productivity conditions. Other sources of calcite input, such as detrital carbonates from the catchment, were unimportant for the studied period.

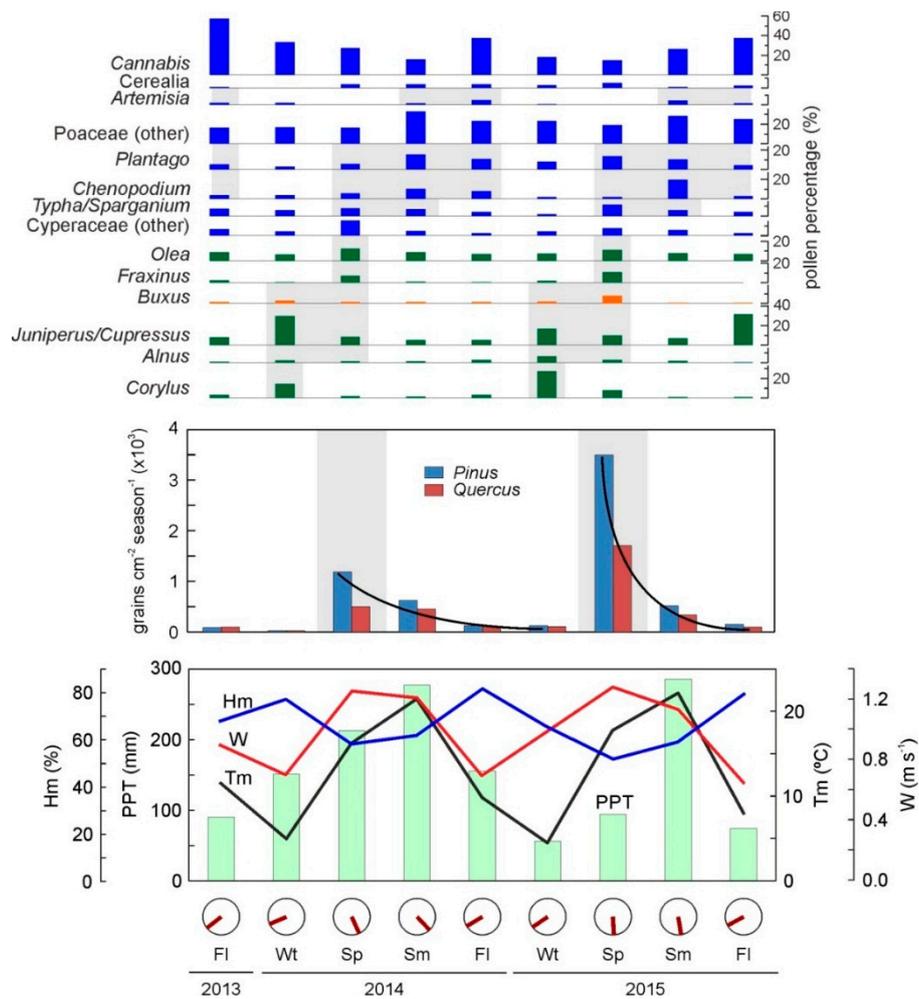
Other seasonal events are precipitation of calcite and OM (Figure 2C). Both variables displayed maximum fluxes in summer to fall. However, calcite precipitation shows the lowest values from winter to spring and was exceeded by OM deposition suggesting a match between the timing of contemporary precipitation of calcite and OM deposition and the alternating couplets of white calcite and brownish organic sublayers of the sediment which would correspond to summer/fall and winter/spring, respectively. These results did not completely back previous paleolimnological inferences that assumed that the light calcite layer was formed in spring/summer and the organic layer was formed in fall/winter [26]. Because of the lack of local or regional modern analogue studies, the authors relied on available literature [17] to explain the observed mechanisms of varve formation.

According to our results, the process of varve forming during the period of study would probably match one of the varve patterns described in the sediment record of Lake Montcortès, once deposited although this remains to be further confirmed. The seasonal calcite-crystal distribution observed in our quarterly sediment traps would produce calcite sub-layering with coarsening upward, i.e., fine-grained calcite crystals deposited in summer and coarse-grained calcite crystals in fall. This texture is often observed in the sedimentary sequence from CE 1350 to 1850 [26]. Modern analogue studies of crystal sizes in calcite sublayers may allow inferring environmental conditions at seasonal resolution, which would be of paleoclimatic significance.

#### 4.2. Is Contemporary Seasonal Deposition of Pollen Consistent with a Two-Layer Varve Model?

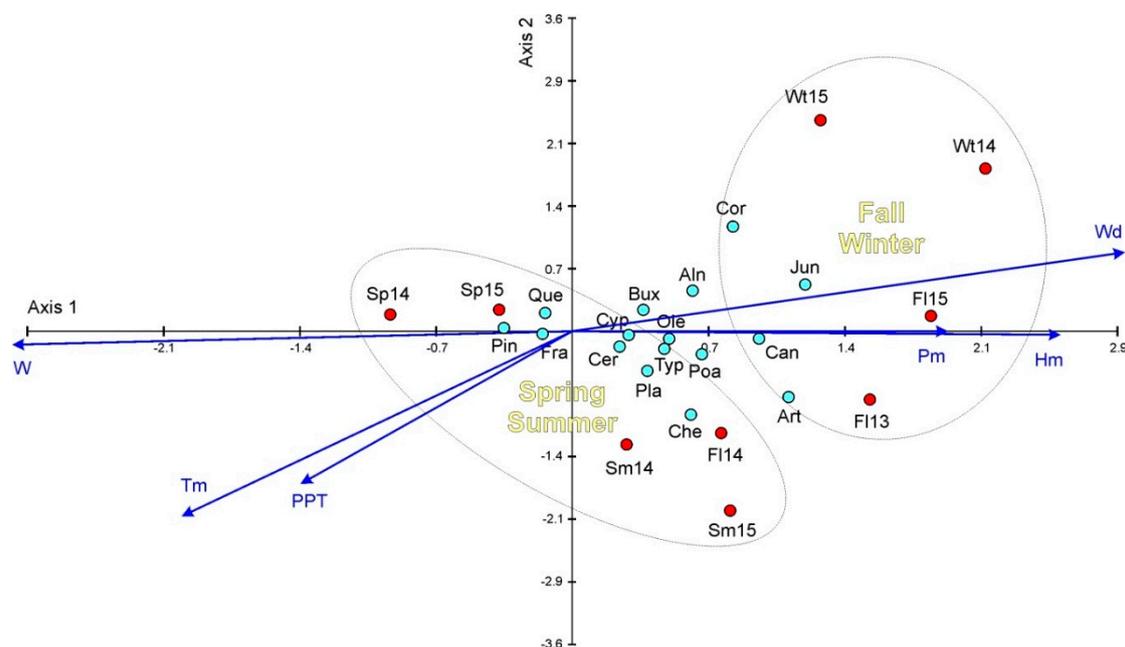
The formation of annually laminated lake sediments relies on seasonal deposition linked to climate and on the related flux of biotic and abiotic matter of multiple autochthonous and allochthonous sources to the sediment. Thus, pollen from the catchment and beyond arrives and is buried in lacustrine sediments. Pollen data have been widely used to reconstruct past climate change and have provided insights into the influence of intra-annual weather conditions to pollination patterns. We now argue that combining pollen data from sediment traps and varved lake sediments with modern meteorological data may produce long, high-resolution ecological time series. An example of what can be achieved with this approach are the continuous, long-term and high-resolution climate series obtained by associating paleoclimate data derived from tree rings and other similar proxies with instrumental climate measurements, at annual and seasonal resolutions [39]. To empirically support this hypothesis, this study is primarily intended to identify modern seasonal depositional patterns of pollen in sediment traps deployed in the varved Lake Montcortès that would be useful for interpreting subfossil pollen records from the same lake.

Pollen maxima were recorded during spring, concurrently with the flowering season of the involved taxa, and preceded the temperature and precipitation maxima in summer by one month (Figure 3). Total pollen influx and taxonomic composition show a strong seasonal signal for the study period, peaking positively during spring/summer and negatively during fall/winter. The major components of the pollen assemblages, *Pinus* (pine) and *Quercus* (oak), dominate the pollen counts and match seasonal trends in temperature and precipitation, with *Pinus* almost tripling *Quercus* percentages in spring/summer but falling to similar levels in fall/winter. As far as the remaining species are concerned, most significant differences were observed in *Plantago*, *Chenopodium*, *Typha/Sparganium*, Cyperaceae, *Fraxinus* and *Juniperus/Cupressus*, which prevailed in the spring/summer assemblage, and *Cannabis* and *Corylus*, which were more abundant in the fall/winter assemblages (Figure 3). Pollen of *Cannabis* (hemp) prevailed during the fall. *Cannabis* is a cultivated plant with pollen being present and fairly abundant in the surroundings of Montcortès for the last 1200 years. Nonetheless, we still have not been able to locate the exact source of that pollen [33,34].



**Figure 3.** Summary of palynological results showing the relationship among the most relevant meteorological parameters (lower panel), the pollen influx of the superabundant pollen types (middle panel) and the percentages of selected pollen types (upper panel). Average temperature (Tm), relative humidity (Hm) and wind velocity (W) are represented by lines, total precipitation (PTT) is represented by bars. The predominant directions of the wind (Wd) are shown as circles (Sp: spring, Sm: summer, Fl: fall, Wt: winter). Pollen percentages were calculated excluding the abundant *Pinus* and *Quercus*. Taxa are ordered by their respective flowering seasons (grey bands) from bottom to top and from left to right. The flowering season of all species of the different genera present in the Montcortès region [40] has been considered. Trees are shown in green, shrubs are in orange and herbs are in blue. Cultivated plants, such as *Cerealia*, *Cannabis* and families including many genera (*Poaceae*, *Cyperaceae*), are located based on their pollen patterns due to the difficulty of establishing a definite flowering season. Redrawn and modified form [19].

Canonical correspondence analysis (CCA) shows the first two axes accounting for 70.7% of total variance. The strongest gradient coincides with axis 1 (56.8% of the total variance), which was highly and positively correlated with relative humidity and atmospheric pressure and negatively correlated with wind velocity (Figure 4). Along this gradient, pollen samples became aligned from spring (left) to winter (right) suggesting a seasonal succession with summer and fall occupying intermediate positions. Pollen taxa were ordered according to the same gradient. The spring/summer group was highly correlated with temperature, precipitation and wind velocity. The fall/winter group was also highly correlated with wind direction from WSW.



**Figure 4.** Canonical correspondence analysis (CCA) biplot using the scores of the first two axes accounting for 70.74% of the total variance. Samples are represented by red dots and pollen taxa (for abbreviations see Figure 3) are represented by blue dots. Samples are identified seasonally as follows: Sp—Spring, Sm—Summer, Fl—Fall, Wt—Winter. Meteorological parameters are abbreviated as in Figure 3: Tm—average temperature, Hm—average relative humidity, Pm—average pressure, W—average wind velocity, PPT—average precipitation, Wd—average wind direction. Pollen types: Aln—*Alnus*, Art—*Artemisia*, Bux—*Buxus*, Can—*Cannabis*, Cer—*Cerealia*, Che—*Chenopodium*, Cor—*Corylus*, Cyp—*Cyperaceae* (other), Fra—*Fraxinus*, Jun—*Juniperus/Cupressus*, Ole—*Olea*, Poa—*Poaceae* (other), Pin—*Pinus*, Pla—*Plantago*, Que—*Quercus*, Typ—*Typha*. Redrawn and modified form [19].

Specific aspects of pollen sedimentation require further attention. For example, a lag in pollen sedimentation was observed between production and deposition throughout the year. This lag can have several causes; for example, the influence of internal water dynamics and resuspension, or the fact that soils can be washed into the lake along several successive months after pollen deposition. With respect to lake internal processes, it is worth mentioning that strong thermal water column stratification occurred from March to November 2014 coinciding with the main pollen production period. This stable stratification could have slowed pollen sedimentation rates, whereas in winter vertical mixing of the water disrupted the thermal stability in January and February 2015, facilitating a release of pollen to the lake bottom several months after their production.

The similarity of sedimentation patterns between *Pinus* and *Quercus* pollen was unexpected, because their respective pollen grains show important morphological differences that make their capabilities for air suspension significantly distinct. However, once the pollen is submerged in the lake water of Lake Montcortès, the settling of both types of pollen was quite similar even during summer, when the thermal stratification was stable. This finding suggests that internal lake dynamics neutralise the effect of such differences and that resuspension or catchment runoff may become more important about pollen sedimentation. In general, the seasonal pattern in pollen deposition coincided well with the suggested two-layered varve model. In summary, pollen analysis was able to identify two well differentiated modern assemblages, one corresponding to spring/summer and the other representing fall/winter. This matches well to the seasonal pattern identified in sedimentological (varve) studies [41]. Thus, utilising appropriate transfer functions supports the hypothesis that the combination of pollen data from sediment traps, varved lake sediments and modern meteorological data may produce long and high-resolution ecological time series.

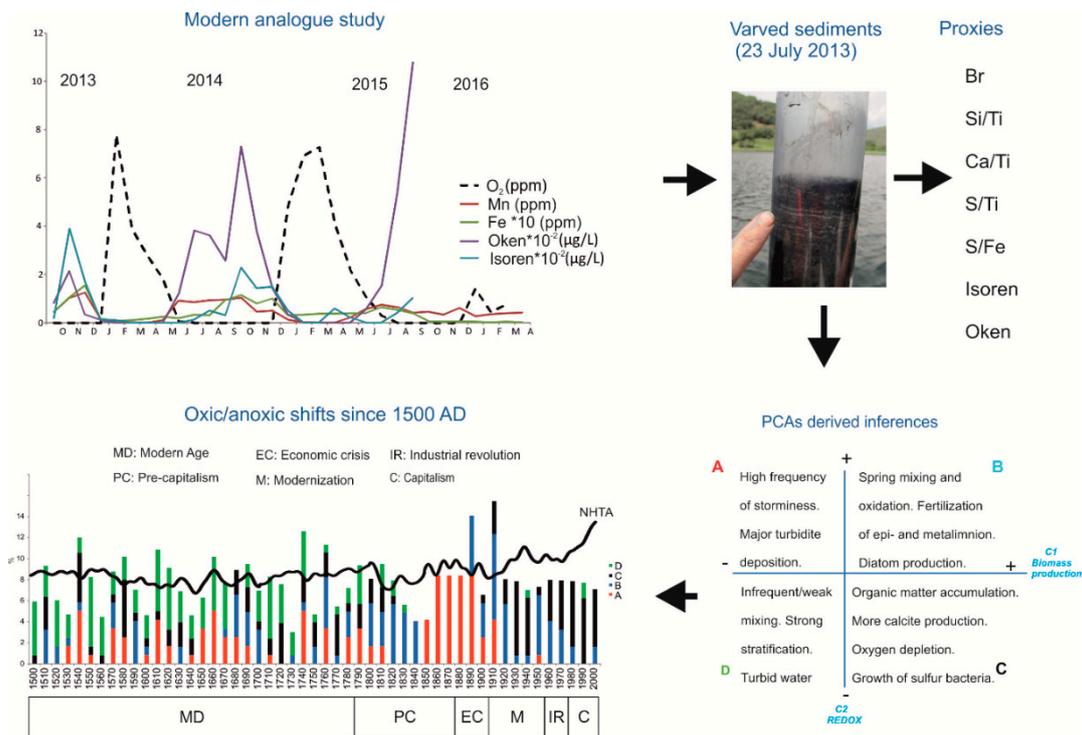
#### 4.3. Revisiting Meromictic Lake Montcortès: Has the Mixing Regime Changed?

Oxygenation of lake water relies on oxygen diffusion from the atmosphere, addition from autochthonous primary production and external inputs of oxygenated water. Lake mixing is the main mechanism, which distributes oxygen throughout the water column and is therefore central for lake health. Mixing regimes can shift with climate changes [42] and human activities [43]. Recently, dissolved oxygen depletion leading to hypoxia or even anoxia has become a matter of concern around the world, and there is a high potential for deterioration under the current global warming [44]. Furthermore, it is suggested that climate change will favour cyanobacteria, which form unpleasant blooms and produce toxins that are harmful to humans [45].

At the same time, obtaining time series of instrumental oxygen records that extend beyond a century is problematic, thus, reducing the possibility of recording long-term changes in oxygen variations that could be associated with climate or human influence. One seminal exception is Lake Zürich, where 50 year long-series of temperature and oxygen profiles corroborated that the increase in the thermal stratification period tends to favour reduction in the homeothermal brake and consequently favours the onset of hypoxia or anoxia [46,47]. Where concurrent series of oxygen and temperature are not available, the combination of proxies of oxic/anoxic conditions with high-resolution paleolimnological records can result in a useful surrogate to assess long-term changes in oxic/anoxic shifts through time.

The varved sediment of LM appears to be highly suitable to provide such high-resolution data. Since redox processes depend on the biogeochemical conditions of lakes and the chemical characteristics of the involved compounds, we firstly conducted a modern analogue study consisting of a monthly survey of main redox indicators, i.e., DO, Fe, Mn and marker pigments [48–51], between October 2013 and October 2016. The same type of marker pigments was also examined in sediments: isorenieratene (Chlorobiaceae) as a tracer of euxinia, okenone (Chromatiaceae) as indicators of anoxia and oscillaxanthin (Oscillatoriales) as a general marker of Cyanobacteria that often points to increased eutrophic conditions. Furthermore, we used selected elemental ratios of the sedimentary record to survey shifts in the oxic/anoxic conditions of the lake over the last 500 years: bromine (Br) as a tracer of organic matter [52], the Ca/Ti and Si/Ti ratios as indicators of biologically mediated calcite and silica production, respectively, and the S/Ti and S/Fe ratios as indicators of the presence of sulphur compounds [53].

Lake Montcortès is reported to be meromictic for years [54]; nonetheless, we observed brief episodes of complete mixing during the winters of 2013 and 2014 that triggered precipitation of Fe and Mn oxides. With the onset of anoxic conditions, precipitated Mn and Fe oxides started to redissolve and sulfur bacteria grew in the hypolimnion under anoxic conditions, when Fe and Mn reached maximum concentrations. The oscillaxanthin was absent in the water column during the sampling period. Such conditions endured over the year 2016, which was a non-mixing year. These results confirmed that redox proxies were working well and provided the information required to properly interpret the sedimentary record. Applying this knowledge to the results obtained from sediment analyses, we were able to distinguish four prevailing situations on the basis of selected redox proxies: (A) years with abrupt and substantial sediment input (turbidites); (B) years with mixing and oxygenation of the water column; (C) years with strong stratification, anoxia, intense activity of sulfur bacteria and increased biomass production; (D) years showing stratification and anoxia, but relatively low biomass production (Figure 5). Interestingly, approximately 45.3% of the years were monomictic years, which mostly occurred in groups of several consecutive years. Meromictic years show a similar pattern. Most (A) years coincide with the climatic instability of CE 1850–1899. The (B) years were rather scattered but were best represented between CE 1820–1849. Most (D) years happened from CE 1500 to 1820, when human activities were locally most intense for the studied period. Almost all (C) years belong to the 20th century. More than 90% of the years with climatic instrumental records belonged to B and C.

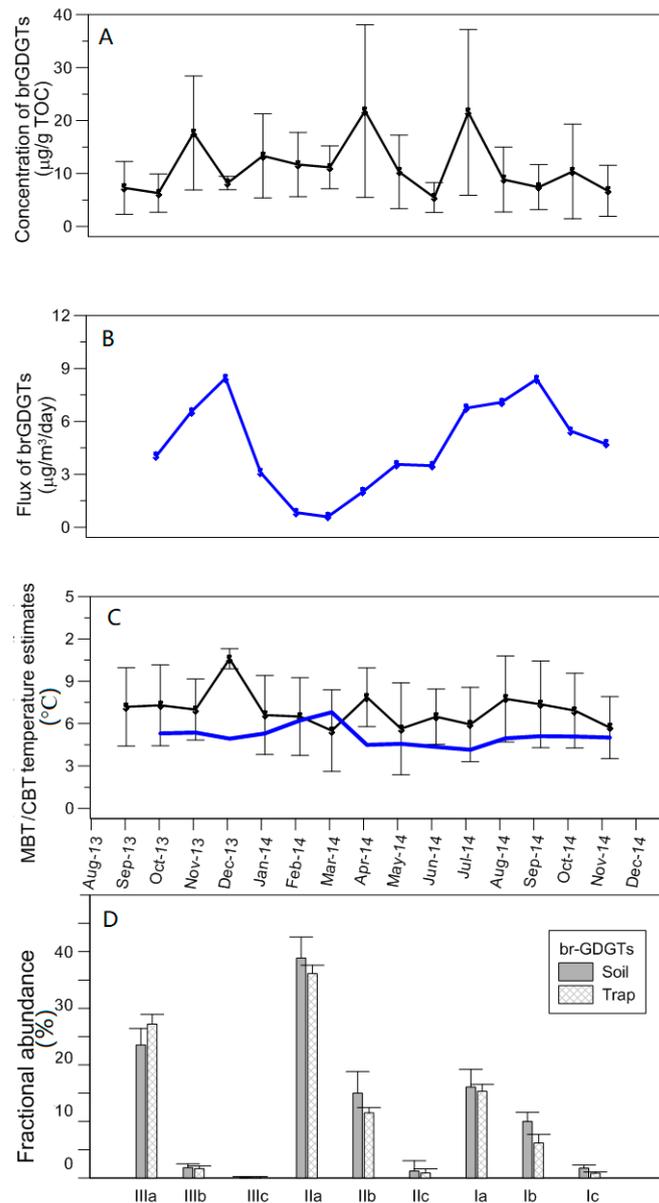


**Figure 5.** Graphical summary of the modern analogue study about oxygen shifts and inferred results: current seasonal variation of mean values of Fe, Mn, O<sub>2</sub> and phototrophic bacteria pigments okenone (oken) and isorenieratene (isoren) in hypolimnetic waters of Lake Montcortès; varved sediments and proxies obtained; graphic synthesis of inferences derived from Principal Component Analyses; decadal evolution of conditions represented by the subsets A, B, C and D, since CE 1500; bars show the percentage of years that fall within a particular decade. NHTA: Northern Hemisphere temperature anomaly [39]. Redrawn and modified form [20].

#### 4.4. Are GDGTs Promising Indicators of Seasonal Temperature Shifts?

Branched glycerol dialkyl tetraethers (brGDGTs) are bacteria derived and widespread lipids of terrestrial and aquatic environments. The global distribution of brGDGTs in soils and peats has been associated with past temperature, and they are therefore used as proxies of mean annual temperature (MAT proxies) in such environments [55]. However, in some regional studies, brGDGTs indices appeared to be influenced by other variables, e.g., precipitation, humidity and soil properties [56]. This proxy was originally calibrated against annual averages of environmental variables. However, a hypothesis arose that these proxy estimates are biased towards particular seasons, because the bioproduction of brGDGTs is enhanced under the most favourable conditions, which occur mostly in summer.

To answer whether soil brGDGT proxy estimates are seasonally biased, the brGDGT distributions and the brGDGT-derived MAT estimates were examined in settling particulate matter (monthly traps) and surface soil samples from the catchment area of Lake Montcortès (MAT: -3.3 to 17.6 °C). No clear-cut seasonal pattern of the brGDGT distribution has been found in soils (Figure 6a), probably because of the slow turnover time in terrestrial environments, which is on timescales of decades or longer. These results confirm previous findings from mid-latitude soils showing that brGDGTs' distribution and some of the brGDGT-derived proxy measurements are relatively stable through the year [57]. However, we found that annual shifts in abundance of brGDGTs were controlled by the variability of specific regional factors, i.e., soil humidity and their pH [13].



**Figure 6.** (A) Concentration of soil brGDGTs, (B) brGDGT flux of particles in the sediment trap at 20 m water depth, (C) brGDGT-based temperature estimates using the MBT/CBT (methylation/cyclisation) proxy in soils (black line, average from three soils in the catchment around the lake) and particles from the sediment trap (blue line). (D) Fractional abundance of brGDGT distribution in soils and sediment traps. Redrawn and modified from [21].

The results record seasonal trends in sediment flux from the catchment area. In the case of particulate matter, heavy rain is the main factor influencing the brGDGT abundance, resulting in seasonal variations of brGDGT flux (Figures 3 and 6b).

In terms of modern analogues, this study offers evidence that the signatures of brGDGTs in depositional environments are representative of soils in the catchment, and that the accuracy of the derived temperature estimates based on methylation/cyclisation (MBT/CBT) proxies in soils depends on soil properties, that in turn depend on regional factors. Additionally, those brGDGT signatures of soils in the catchment probably represent average environmental conditions over decades or longer, and this observation means that any derived proxy reconstructions can be used only to infer variability in environmental variables over the same timescales. The absence of seasonality in brGDGT proxies from the sediment traps Lake Montcortès is due to the lack of significant “in situ” lacustrine production

of brGDGTs (Figure 6c). The most straightforward explanation is that the main sources of brGDGTs are catchment soils, and that their non-seasonal signals are transferred to the settling particles, which have a distribution of brGDGTs that appear to be a weighted mean of soil signals (Figure 6c). In addition, similar patterns of brGDGT fractional abundance in soils and sediment traps confirm soil-related sources to the particles in sediment traps (Figure 6d). It remains unclear if downcore variability in brGDGTs at an annual scale will also capture changes derived from in situ production, and if derived brGDGT proxies will be suitable to build high-resolution MAT reconstructions.

#### 4.5. From Contemporary Phytoplankton to Subfossil Pigments, What Can We Learn about Community Changes?

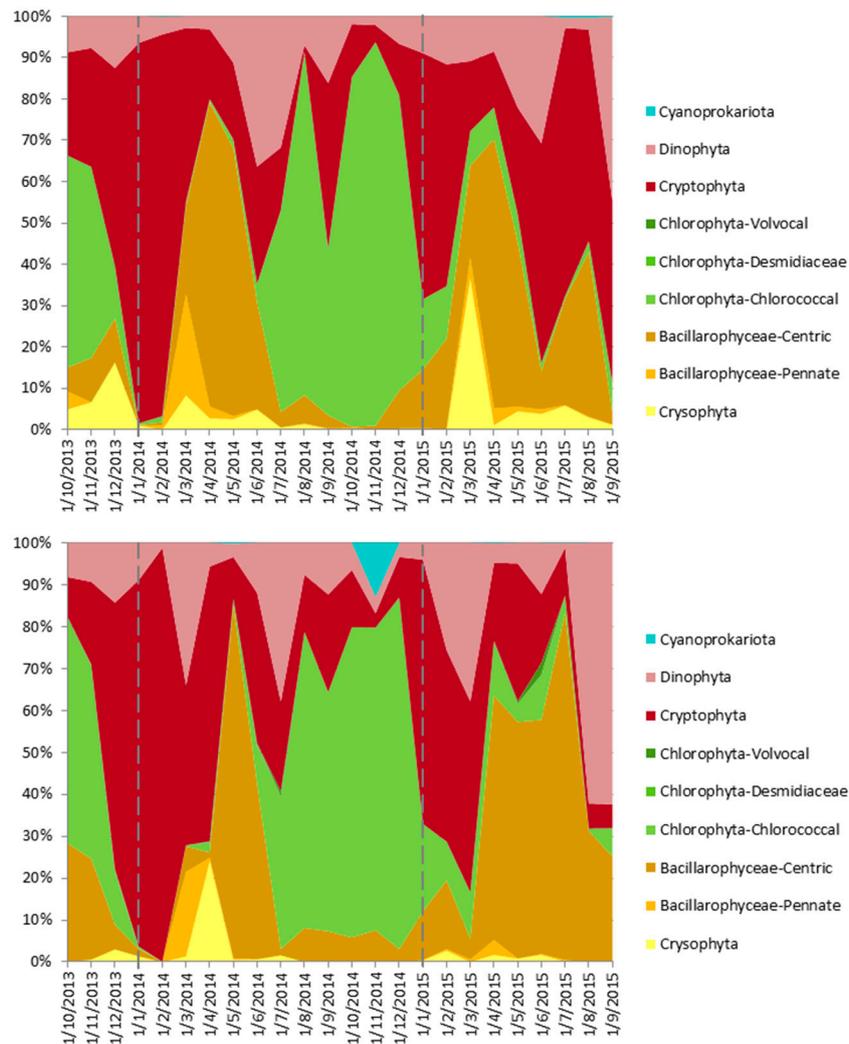
In general, qualitative and quantitative relationships between modern climatic variables, aquatic primary producers and their pigments and subfossil marker pigments of the sediment have received little attention despite their potential to build reliable climatic proxies. To help fill this gap, we performed a modern analogue study to determine how phytoplankton and marker pigment information compare with the sedimentary record. For this purpose, we applied an analogue matching (AM) technique, which is concerned about identifying contemporary sites that most closely match the species assemblage identified in the past [7].

We assessed and compared the annual cycle of phytoplankton in the epi- and metalimnion and of marker pigments in the epi-, meta- and hypolimnion, and also in a sediment trap deployed at 20 m water depth. Redundancy Analysis (RDA) was applied to identify potential associations between the phytoplankton taxa identified by microscopy. Then, we performed a similarity analysis in order to examine expected bias between modern and subfossil samples (CE 1493–2013) in terms of presence or absence of common pigment markers.

The total biovolume of phytoplankton depicted a regular annual cycle with growing peaks in early spring and summer and low biomass in winter (Figure 2B). Phytoplankton biovolume of the metalimnion was highest in summer ( $>6 \times 10^6 \mu\text{m}^3/\text{mL}$ ). Centric Bacillariophyceae and Chlorophytes follow one another as the dominant taxa in 2013–2014, whereas smaller centric Bacillariophyceae took over during 2014–2015 growing periods (Figure 7).

The RDA analysis allowed to identify marker pigments more tightly associated with each taxonomic group (Figure 8). Chlorophyta, Dinophyta, Cyanoprokaryota (Cyanobacteria and Cyanophyta), centric Bacillariophyceae, Volvocales and Cryptophyta biovolumes explain a significant percentage of variance in marker pigment concentration, while the percentage variance explained by the other taxonomic groups was insignificant. Each taxonomic group was associated with specific marker pigments. The biovolume of Chlorophyta was associated with the marker pigments neoxanthin, lutein, chlorophyll-b and zeaxanthin, the biovolume of Dinophyta mainly with peridinin, diadinoxanthin, fucoxanthin, Chl-c1 and c2, although Chl-c2 was also partially associated with the biovolume of Cryptophyta. The biovolume of Cryptophyta was mainly associated with alloxanthin and a-carotene, both partially explained by pennate Bacillariophyceae. We know from the literature that some of the pigments associated with Dinophyta are also present in Bacillariophyceae (e.g., fucoxanthin, diadinoxanthin, Chl-c1 and Chl-c2), which is coherent with the observed RDA biplot association. Cyanobacteria biovolume was associated with myxoxanthophyll-like, while association with marker pigments of phototrophic bacteria is due to co-occurrence in the samples with these groups. These data are essential to identify pigments with a higher potential to be used as modern analogues in the sediment record.

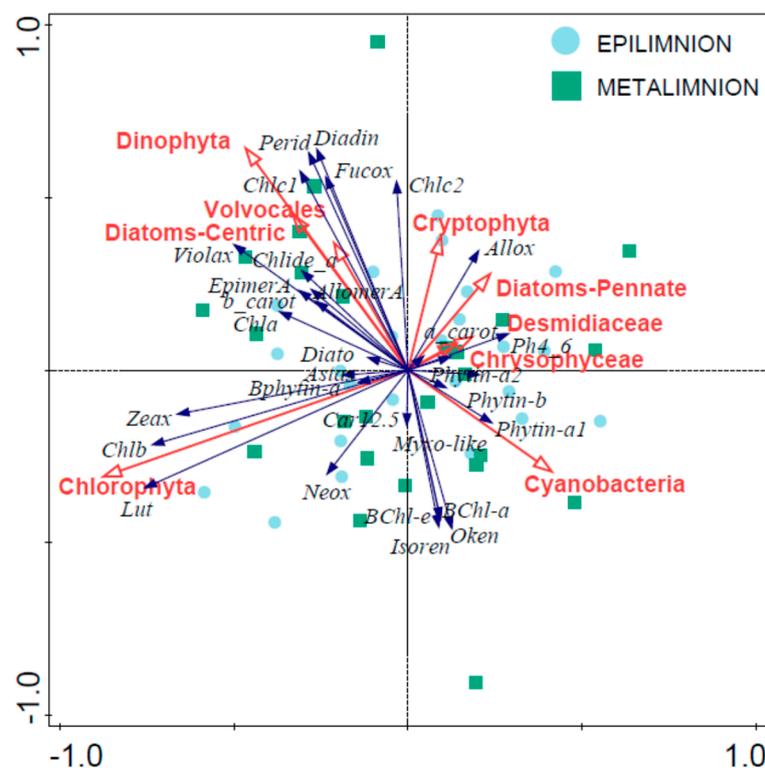
All this information is relevant to better identify pigments from sediment traps and use them as modern analogues for the sediment record following taxonomic affinity criteria and preservation.



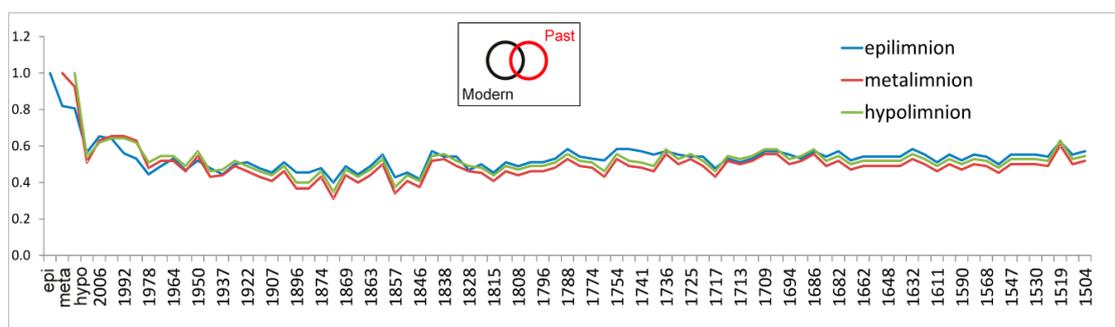
**Figure 7.** Taxonomic phytoplankton groups with their biovolume and relative dominance (%) in the epi- (**upper graph**) and the metalimnion (**lower graph**) from October 2013 to September 2015 (abscissa).

The concentration of most representative marker pigments was calculated for the entire water sampling period (2013–2015), in order to compare their spatial variations between epi-, meta- and hypolimnion, as well as with their deposition in the sediment trap (Figure S1, Supplementary Materials). Marker pigments of Bacillariophyceae (fucoxanthin: Figure S1c) and Dinophyta (diadinoxanthin: Figure S1d) were produced and deposited in higher amounts in spring and summer while Chlorophyta marker pigments (Chl-b, lutein and zeaxanthin: Figure S1e,f,h) were produced mainly in fall, and marker pigments of Cryptophyta (alloxanthin: Figure S1g) were produced throughout the year with the exception of fall. Zeaxanthin is a marker pigment also indicative of Cyanobacteria and decoupled from parent marker pigments of Chlorophyta during summer, it indicates a higher contribution of Cyanobacteria during summer months (Figure S1). Marker pigments of photosynthetic sulphur bacteria were only found in the hypolimnion during summer and fall (Figure S1i,j). Most marker pigments from the water column were also identified in the sediment record. A list of the marker pigments co-occurring in the water column and sediment record is given in Table S1 (Supplementary Materials). Their taxonomic affinities are also shown and inform about their likely biological sources, which are consistent with the taxonomic phytoplankton groups of the epi- and metalimnion (Figure 7). To gauge the similarity between water column and sediment samples we applied the Sorensen index (S) on a similarity matrix built with binary data. This very simple similarity index provides a greater “weight” to species common to the samples than to those found in only one sample. According to S,

epi-, meta- and hypolimnetic samples resemble at  $\geq 80\%$ , whereas meta and hypolimnetic samples resemble each other relatively more (92%), likely because of lower oxidation rates during anoxic conditions of the hypolimnion. Interestingly, when compared with sediment samples of the top of the core (CE 2007–2013 E), *S* diminished abruptly to 0.5, that is, the bias was 50% (Figure 9). This decrease was followed by a slower decrease of *S*, until values of 30% (circa 1850) and its posterior stabilisation at values of 40–60% that lasted circa two centuries. A Venn diagram highlights that past communities only have a partial modern analogue (Figure 9) [5]. These results indicate that approximately 50% of the marker pigments in the water column were destroyed between deposition and permanent burying in the first centimetres of the sediment record, whereas the remaining 40–60% were accurately represented over the period of study. This pattern is roughly consistent with the three phases of sediment loss proposed by [48]: (1) rapid oxidation, enzymatic metabolism and digestion by herbivores through the water column (half-life  $T_{1/2}$  = days) would account for a breakdown of pigments while they sink, (2) slower post-depositional loss in surface sediments would result from structural rearrangements, release of labile compounds and further oxidation ( $T_{1/2}$  = years) and (3) once buried pigment degradation would have continued at a very slow pace ( $T_{1/2}$  = centuries).



**Figure 8.** Redundancy analysis (RDA) between phytoplankton taxa (biovolume;  $\text{mm}^3 \cdot \text{L}^{-1}$ ) and marker pigments (concentration;  $\mu\text{g} \cdot \text{L}^{-1}$ ). Red arrows are explanatory variables (53% of the variance in pigment concentration was explained by phytoplankton taxa), blue arrows are response variables (pigments). Diadin: diadinoxanthin; Fucox: fucoxanthin; Chlc2: chlorophyll-c2; Allox: alloxanthin; a-carot: a-carotene; Ph4\_6: unknown phorbins; Phytin-a1,a2: phaeophythin-a1, a2; Phytin-b: phaeophythin-b; Car12.5: unknown carotenoid; Myxo-like: myxoxanthophyll-like; BChl-a: bacteriochlorophyll-a; BChl-e: bacteriochlorophyll-e; Oken: okenone; Isoren: isorenieratene; Neox: neoxanthin; Lut: lutein; Chlb: chlorophyll-b; Zeax: zeaxanthin; Bphytin-a: bacteriophageophythin-a; Astax: astaxanthin; Diato: diatoxanthin; Chla: chlorophyll-a; b-carot: b-carotene; EpimerA: Chl-a epimer; AllomerA: Chl-a allomer; Violax: violaxanthin; Chlide\_a: chlorophyllide-a; Chlc1: chlorophyll-c1; Perid: peridinin.



**Figure 9.** Comparison of Sorensen's similarity index (S) between modern and sediment samples. A Venn diagram comparing modern and past pigment assemblages is shown in the upper right corner. Epi-, meta-, hypo- refer to current in-depth layers of the water column. Abcissa: dates inform about the age of the sediment samples, Ordinate: S values.

Finally, Table S2 (Supplementary Materials) shows which marker pigments were found to be exclusive from present-day samples (6) and which were found only in subfossil samples (7). Some of the unspecific chlorophyll derivatives are produced under distinct environmental conditions and by different mechanisms with time, chiefly through losses of  $Mg^{+2}$ , herbivory, viral attack and enzymatic catalysis [48]. Noteworthy, however, is the presence of four Cyanobacterial markers in the subfossil record that were not present in modern samples, suggesting either a detection limit for the identification of these pigments in the water column or that past lake conditions were favourable to a high diversity of Cyanobacterial groups including  $N_2$ -fixing. In fact, massive blooms of filamentous *Planktothrix rubescens* De Candolle ex Gomont have been reported to have flourished in Lake Montcortès in the 1970s [54]. However, based on microscopical counts and marker pigment detection, Cyanobacteria seem to constitute only a low percentage of the phytoplankton currently thriving at Lake Montcortès (Figure 6), an issue that needs further confirmation.

## 5. Discussion, Conclusions and Guidelines for Future Research

To date, most paleoenvironmental reconstructions for Lake Montcortès have been performed using conventional methods and models, which were originally thought for lower resolution studies and higher latitudes. They are therefore constrained to the supra-decadal level. However, it is clear that the sublayers of varves host invaluable, high resolution information that still has to be retrieved. Our modern analogue studies provide detailed information about fine-tuned processes that take place at annual and sub-annual scales and show how a suite of widely used proxies respond at the same temporal resolution. We expect that each proxy is physically deposited in a varve sublayer in temporal correspondence with the processes it represents. Being able to put this exact match in evidence is expected to boost the interpretative power of future high-resolution paleoenvironmental reconstructions.

The main contribution of this review is to show the advantages and limitations of a multiproxy modern-analogue approach in Lake Montcortès as a case study. This has not been published previously in any other papers. There exist other studies with a similar approach, e.g., lake Zabiński (Poland) [58] or Lake La Cruz (Spain) [59–62], but results in every case are different because sedimentary and biogeochemical processes differ among lakes. Obviously, results of one particular lake may not be directly applicable at other lakes. Our results demonstrate that current lacustrine processes of Lake Montcortès involving calcite precipitation, phytoplankton and bacterial growth, oxygenation of the water column and pollen production, have a clear seasonality and also a year-to-year variability, depending on subtle interannual climatic variations. Therefore, it is reasonable to expect finding the corresponding fingerprints of these processes at the varve (sublayer) levels. Interestingly, the brGDGT production in soils is not seasonal, while the particle flux into the lake is. The similarity of brGDGT distributions indicated the same sources of brGDGTs in soils and lakes. The brGDGT-estimated

temperature reflected the interannual average conditions from the soil records. In order to provide an integrated overview of the basic functioning of Lake Montcortès related to our modern analogue studies, a synthesis of the main processes and their spatial and temporal course is presented (Figure 10). This figure is intended to serve as a guide for high resolution paleoecological and paleoenvironmental reconstructions and derived inferences in lake Montcortès and other similar lakes. It shows how limnological variables and processes give rise to the proxies that will be used in these reconstructions, the transit of the latter from present to past conditions, and where in the varve structure would these proxies be found, according to the season where they are produced and settled down (time-lag = 0 is assumed). In this figure, the limnological variables under study are listed (column 1); moreover, the contemporary processes (column 4) that occur in the lake or lake catchment over the year (columns 2, 3). Columns 5 to 8 show the seasons when these processes occur (blue) and when they are more intense (dark blue). It is also indicated which varve sublayer is representative of each season. White and a dark sublayer correspond to summer and winter, respectively, dark-white transitions to spring and white-dark transitions to fall. Column 9 displays degradation and diagenetic mechanism that are expected to act upon the limnological variables inside the sediment and column 10 the derived proxies. Column 11 suggests which varve sublayers should be targeted to retrieve these proxies for high resolution reconstructions, accepting that one single varve contains all seasonal information needed, i.e., in correspondence with white and dark sublayers and their transitions. We also assume that highly precise sampling methods are available in every case. This is an especially sensitive issue in the case of Lake Montcortès where varves are relatively thin, e.g., total mean varve thickness averages 1.3 mm between the 14th and 20th century, with calcite sublayers 0.35 mm and organic sublayers 0.34 mm [26], and probably in other lakes as well. Note for example that by now, neither diatoms nor pollen or pigments could be extracted from a single varve and even less from a sublayer, due to methodological limitations.

Modern-analogue variables	Catchment	Lake	Current process	Sp (tr d-w)	Sm (w)	Fl (tr w-d)	Wt (d)	Transformation in the sediment (diagenesis)	Sedimentary proxy	Expected main target sublayers
Calcite saturation index	x	x	Endogenic Ca precipitation, primary production.					Calcite deposition and accumulation.	Ca/Ti, calcite laminations.	w; tr w-d
Large calcite crystals		x	Authigenic formation of calcite crystals, low T°C.					Accumulation and growth.	Larger calcite crystals.	tr w-d; d
Small calcite crystals			Biogenic formation of calcite crystals, high T°C.					Accumulation and growth.	Smaller calcite crystals.	tr d-w; w
Particulate organic C (POC)	x	x	Lacustrine primary production.					Organic carbon accumulation/oxydation.	Br, S/Ti	w; tr w-d
Phytoplankton biovolume		x	Phytoplankton growth and succession.					Degradation.	Subfossil pigments.	w; tr w-d
Diatom species		x	Diatom blooms.					Accumulation of frustules.	Subfossil diatoms.	tr d-w
Oxygen		x	O <sub>2</sub> consumption and supply.					O <sub>2</sub> consumption.	Oxidised/reduced mineral form	tr d-w; tr w-d
O <sub>2</sub> , Mn,Fe	x	x	Hypolimnetic oxidation, mixing.					Precipitates of Mn, Fe oxides/hydroxides.	Fe/Mn, Fe/Ti, absence of anaerobic pigments.	d
O <sub>2</sub> , Mn,Fe	x	x	Hypolimnetic reduction, anoxia.					Redissolution of Mn, Fe oxides/hydroxides	Fe/Mn, Mn/Ti, presence of anaerobic bacterial pigments.	w; tr w-d
<b>POLLEN</b>										
<i>Pinus</i>	x		Flowering season					Partial degradation	Pollen abundance	tr d-w; w
<i>Plantago</i>	x		Flowering season					Partial degradation	Pollen abundance	w
<i>Chenopodium</i>	x		Flowering season					Partial degradation	Pollen abundance	w
<i>Typha/Sparganium</i>		x	Flowering season					Partial degradation	Pollen abundance	tr d-w; w
<i>Fraxinus</i>	x		Flowering season					Partial degradation	Pollen abundance	tr d-w
<i>Juniperus/Cupressus</i>	x		Flowering season					Partial degradation	Pollen abundance	tr w-d; d
<i>Camnabis</i>	x		Flowering season					Partial degradation	Pollen abundance	tr w-d; d
<i>Corylus</i>	x		Flowering season					Partial degradation	Pollen abundance	d
Total pollen	x		Flowering season					Partial degradation	Pollen abundance	tr d-w; w
<b>LIPIDS</b>										
brGDGT in soils	x		Bioproduction of brGDGT						brGDGT	
brGDGT in traps		x	brGDGT drainage					Accumulation and degradation	brGDGT	tr w-d
<b>PRIMARY PRODUCERS</b>										
Chlorophyta biovolume		x	Pigment production					Degradation and oxidation	Production of neox, lut, chl-b, zeax.	w; tr w-d
Dinophyta biovolume		x	Pigment production					Degradation and oxidation	Production of perid, diadinox, fucox, Chl-c1 and c2.	tr d-w; w; tr w-d; d
Cryptophyta biovolume		x	Pigment production					Degradation and oxidation	Production of allox, a-carot,	all sublayers
Bacillariophyta		x	Pigment production, silicate consumption					Degradation and oxidation	Production of allox, a-carot, fucox, diadinox, Chlc1, Chlc2. Diatom frustules.	tr d-w
Cyanobacteria		x	Pigment production					Degradation and oxidation	Production of myxo-like.	tr w+d
Phototrophic bacteria		x	Pigment production					Degradation and oxidation	Production of oken, isoren.	w; tr w-d

**Figure 10.** Synthesis of main modern analogue variables and processes at Lake Montcortès, derived proxies and their expected target sublayers. White (w); dark (d): white-dark transitions (w-d tr) and dark-white transitions (d-w tr) between sublayers. Sp: spring, Sm: summer, Fl: Fall, Wt: winter.

Finally, we provide some guidelines for future research at Lake Montcortès and similar lakes.

- i. Calcite: There is evidence that a temperature increase enhances the amount of calcite precipitation and flux to the sediment. This relationship may be an important factor, when considering the role of calcite as a sink of atmospheric CO<sub>2</sub> in the context of global warming and needs further examination. On the other hand, if the size of endogenic calcite crystals varies seasonally, inferences at the sub-annual scale may be derived from calcite crystals contained in sublayers of varves.
- ii. These hypotheses encourage the execution of further modern analogue studies. Hence, three improvements are suggested: (1) increase the frequency and duration of sampling, in order to better document shifts in calcite precipitation as related to the current warming trend; (2) install a meteorological station in the lake's catchment, in order to capture local temperature and other meteorological variables that condition water temperature; (3) perform a detailed survey of calcite crystal features retrieved from seasonal traps and from varve sublayers to precisely determine their origin and diagnostic characteristics.
- iii. Pollen: Two advantages of pollen studies are (1) the ability of identifying and characterizing seasonal layers even in the absence of varves and (2) the possibility of recording interannual variability and associated meteorological drivers.
- iv. Oxygen: Even though our approach cannot provide estimations of past oxygen concentrations, it is a useful tool to connect and compare the dynamics of past and present oxic and anoxic events with annual resolution. However, if past oxygen concentrations are to be inferred, a more specific modern analogue study is required. Perhaps monthly and multiannual monitoring of the evolution of hypolimnetic oxygen concentrations and a concurrently examination of the content of Fe and Mn oxides in surface sediment samples, catches mixing and meromictic years. It should then be possible to establish appropriate transfer functions to estimate dissolved oxygen from most related proxies. If successful, truly long-term oxygen time series linking estimated and instrumental data could be established.
- v. Cyanoprokariota assemblages lack a modern analogue, since they are nearly absent today, whereas they were abundant, diverse and pervasive until, at least, the 1970s [54]. This observation is surprising because, to our knowledge, there have been few changes in land use or lake exploitation that could have affected Lake Montcortès' trophic conditions. In contrast, we expect Cyanoprokariota to play a prominent role during the coming warmer years, when Lake Montcortès becomes more intensely stratified. Cyanobacteria marker pigments (myxoxanthophyll and zeaxanthin) have been detected in the water column during monitoring, the lack of correspondence with microscopical counts may be due to the low cell size of species involved, but this will need further studies. Therefore, considering the known deleterious effects of Cyanobacteria on aquatic ecosystems and human health, it would be advisable to continue present-day monitoring. The results would aid in obtaining a long-term picture of cyanobacterial successions and importance and allow deriving future scenarios, in the context of global warming.
- vi. brGDGT production in the soils of the lake catchment show no seasonality. However, in sediment trap particles, the brGDGT flux presents a clear seasonality. The seasonal variations of flux rates confirm transport variations from the catchment rather than in situ production of brGDGT in the lacustrine environment. The MBT/CBT temperature estimates indicate that brGDGT signatures in the sediment traps show a mixed signature and originate from surrounding soils. These results suggest that any inferences made from the high-resolution record must be made with caution.
- vii. From a methodological point of view, cylindrical traps with seasonal or quarterly recovery yield coherent results in all cases, i.e., total suspended solids, calcite crystals, pollen and spores and particles for brGDGT extractions. However, it is recommended that additional samples of surface sediments at the end of sampling years should be taken, to assess post-depositional transformations of these proxies. In our case, surface sediment samples would have provided

complementary information, such as total fluxes to the sediment and subsequent accumulation, interactions at the sediment-water interface and early diagenesis. For example, in the case of calcite precipitation as related to varve formation, initial data of organic matter content may be needed to estimate the degree of sediment compaction with time [63] and for a full understanding of structure and thickness of varve sublayers at any depth. In the end, all this information allows a better connection and comparison between contemporary proxies and their modified version after being buried in the sediment, making the modern analogue technique more powerful.

- viii. Sometimes, modern analogue studies performed at a seasonal or annual frequency cannot be fully exploited for the interpretation of sedimentary records, because of a mismatch in resolution between present-day and past samples. This mismatch occurs when a relatively large weight or volume of sediment is needed to extract enough material for proxy analyses, because contiguous varves are too thin and/or must be joined into one sample. In doing so, the resolution of the samples decreases. With time and targeted effort, this disadvantage will hopefully be overcome with the advent of new technologies. In the field of marker pigments, hyperspectral image spectroscopy is progressing quickly and offers a non-destructive and inexpensive approach that permits high resolution. Hyperspectral image spectroscopy is being used in lake sediments, e.g., for concentrations of sedimentary bacteriopheophytin “a”, based upon diagnostic spectral properties with high spatial and temporal resolution [64]. A good match of the resolutions of modern and past studies is indispensable to successfully connect both and to better understand the evolution of ecosystems with time.

**Supplementary Materials:** The Supplementary Materials is available online at <http://www.mdpi.com/2571-550X/3/1/1/s1>. Figure S1: Comparison of most representative modern marker pigments from the epi-, meta- and hypolimnion as well as their deposition in the sediment trap, on the basis of annual values of the period 2013–2015; Table S1: Marker pigments co-occurring in the water column and sediment record and their taxonomic affinities for lake Montcortès; Table S2: Marker pigments and their taxonomic affinities for Lake Montcortès.

**Author Contributions:** T.V.-V. conceived and wrote the paper. T.B., V.R., M.d.C.T., M.C., A.R.-M., J.G., J.P.C. and P.L. contributed by refining the text and with data, ideas and figures. E.S., M.d.C.T., N.C., J.G., S.G.-P., P.L., S.G., J.P.C. and T.B. carried out fieldwork and laboratory analyses. J.S. and N.P.-Z. advised on climatic aspects and contributed with meteorological data. All co-authors reviewed the paper. All authors have read and agreed to the published version of the manuscript.

**Funding:** Research funding was granted by the Spanish Ministry of Economy and Competitiveness (MINECO/FEDER) with the projects MONT-500, ref. CGL2012-33665; GLOBALKARST, ref. CGL2009-08145), the Institute of Catalan Studies with the projects PIRIMOD and POLMONT, and the Catalan University and Research Management Agency (AGAUR) with the projects 2014 SGR 1207, 2017 SGR 1116).

**Acknowledgments:** We acknowledge the Busseing Pallars Company, the Council of Baix Pallars and the cultural association Lo Vent de Port for their direct involvement in the project and their continuous support.

**Conflicts of Interest:** The authors declare no conflict of interest.

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